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(54) Title: PEPTIDE-BASED COMPOUNDS

(57) Abstract: The invention relates to new peptide-based compounds for use as diagnostic imaging agents or as therapeutic agents wherein the agents comprise targeting vectors which bind to integrin receptors.

Peptide-based compounds

Field of invention

The present invention relates to new peptide-based compounds and their use in therapeutically effective treatments as well as for diagnostic imaging techniques. More specifically the invention relates to the use of such peptide-based compounds as targeting vectors that bind to receptors associated with angiogenesis, in particular integrin receptors, e.g. the αvβ3 integrin receptor. Such contrast agents may thus be used for diagnosis of for example malignant diseases, heart diseases, endometriosis, inflammation-related diseases, rheumatoid arthritis and Kaposi's sarcoma. Moreover such agents may be used in therapeutic treatment of these diseases.

Background of invention

New blood vessels can be formed by two different mechanisms: vasculogenesis or angiogenesis. Angiogenesis is the formation of new blood vessels by branching from existing vessels. The primary stimulus for this process may be inadequate supply of nutrients and oxygen (hypoxia) to cells in a tissue. The cells may respond by secreting angiogenic factors, of which there are many; one example, which is frequently referred to, is vascular endothelial growth factor (VEGF). These factors initiate the secretion of proteolytic enzymes that break down the proteins of the basement membrane, as well as inhibitors that limit the action of these potentially harmful enzymes. The other prominent effect of angiogenic factors is to cause endothelial cells to migrate and divide. Endothelial cells that are attached to the basement membrane, which forms a continuous sheet around blood vessels on the contralumenal side, do not undergo mitosis. The combined effect of loss of attachment and signals from the receptors for angiogenic factors is to cause

the endothelial cells to move, multiply, and rearrange themselves, and finally to synthesise a basement membrane around the new vessels.

Angiogenesis is prominent in the growth and remodelling of tissues, including wound healing and inflammatory processes. Tumors must initiate angiogenesis when they reach millimetre size in order to keep up their rate of growth. Angiogenesis is accompanied by characteristic changes in endothelial cells and their environment. The surface of these cells is remodelled in preparation for migration, and cryptic structures are exposed where the basement membrane is degraded, in addition to the variety of proteins which are involved in effecting and controlling proteolysis. In the case of tumours, the resulting network of blood vessels is usually disorganised, with the formation of sharp kinks and also arteriovenous shunts. Inhibition of angiogenesis is also considered to be a promising strategy for antitumour therapy. The transformations accompanying angiogenesis are also very promising for diagnosis, an obvious example being malignant disease, but the concept also shows great promise in inflammation and a variety of inflammation-related diseases, including atherosclerosis, the macrophages of early atherosclerotic lesions being potential sources of angiogenic factors. These factors are also involved in re-vascularisation of infarcted parts of the myocardium, which occurs if a stenosis is released within a short time.

Further examples of undesired conditions that are associated with neovascularization or angiogenesis, the development or proliferation of new blood vessels are shown below. Reference is also made in this regard to WO 98/47541.

Diseases and indications associated with angiogenesis are e.g. different forms of cancer and metastasis, e.g. breast, skin, colorectal, pancreatic, prostate, lung or ovarian cancer.

Other diseases and indications are inflammation (e.g. chronic), atherosclerosis, rheumatoid arthritis and gingivitis.

Further diseases and indications associated with angiogenesis are arteriovenous alformations, astrocytomas, choriocarcinomas, glioblastomas, gliomas, hemangiomas (childhood, capillary), hepatomas, hyperplastic endometrium, ischemic myocardium, endometriosis, Kaposi sarcoma, macular degeneration, melanoma, neuroblastomas, occluding peripheral artery disease, osteoarthritis, psoriasis, retinopathy (diabetic, proliferative), scleroderma, seminomas and ulcerative colitis.

Angiogenesis involves receptors that are unique to endothelial cells and surrounding tissues. These markers include growth factor receptors such as VEGF and the Integrin family of receptors. Immunohistochemical studies have demonstrated that a variety of integrins perhaps most importantly the α_v class are expressed on the apical surface of blood vessels [Conforti, G., et al. (1992) Blood 80: 37-446] and are available for targeting by circulating ligands [Pasqualini, R., et al. (1997) Nature Biotechnology 15: 542-546]. The $\alpha 5\beta 1$ is also an important integrin in promoting the assembly of fibronectin matrix and initiating cell attachment to fibronectin. It also plays a crucial role in cell migration [Bauer, J. S., (1992) J. Cell Biol. 116: 477-487]

as well as tumour invasion and metastasis [Gehlsen, K. R., (1988) J. Cell Biol. 106: 925-930].

The integrin αvβ3 is one of the receptors that is known to be associated with angiogenesis. Stimulated endothelial cells appear to rely on this receptor for survival during a critical period of the angiogeneic process, as antagonists of the αvβ3 integrin receptor/ligand interaction induce apoptosis and inhibit blood vessel growth.

Integrins are heterodimeric molecules in which the α- and β-subunits penetrate the cell-membrane lipid bilayer. The α-subunit has four Ca^{2+} binding domains on its extracellular chain, and the β-subunit has a number of extracellular cysteine-rich domains.

Many ligands (eg. fibronectin) involved in cell adhesion contain the tripeptide sequence arginine-glycine-aspartic acid (RGD). The RGD sequence appears to act as a primary recognition site between the ligands presenting this sequence and receptors on the surface of cells. It is generally believed that secondary interactions between the ligand and receptor enhance the specificity of the interaction. These secondary interactions might take place between moieties of the ligand and receptor that are immediately adjacent to the RGD sequence or at sites that are distant from the RGD sequence.

RGD peptides are known to bind to a range of integrin receptors and have the potential to regulate a number of cellular events of significant application in the clinical setting. (Ruoslahti, J. Clin. Invest., 87: 1-5 (1991)). Perhaps the most widely studied effect of RGD peptides and

mimetics thereof relate to their use as anti-thrombotic agents where they target the platelet integrin GpIIbIIIa.

Inhibition of angiogenesis in tissues by administration of either an αvβ3 or αvβ5 antagonist has been described in for example WO 97/06791 and WO 95/25543 using either antibodies or RGD containing peptides. EP 578083 describes a series of mono-cyclic RGD containing peptides and WO 90/14103 claims RGD-antibodies. Haubner et al. in the J. Nucl. Med. (1999); 40: 1061-1071 describe a new class of tracers for tumour targeting based on monocyclic RGD containing peptides. Biodistribution studies using whole-body autoradiographic imaging revealed however that the ^{125}I-labelled peptides had very fast blood clearance rates and predominantly hepatobiliary excretion routes resulting in high background.

Cyclic RGD peptides containing multiple bridges have also been described in WO 98/54347 and WO 95/14714. Peptides derived from in vivo biopanning (WO 97/10507) have been used for a variety of targeting applications. The sequence CDCRGDCFC (RGD-4C), has been used to target drugs such as doxirubicin (WO 98/10795), nucleic acids and adenoviruses to cells (see WO 99/40214, WO 99/39734, WO 98/54347, WO 98/54346, US 5846782). Peptides containing multiple cysteine residues do however suffer from the disadvantage that multiple disulphide isomers can occur. A peptide with 4 cysteine residues such as RGD-4C has the possibility of forming 3 different disulphide folded forms. The isomers will have varying affinity for the integrin receptor as the RGD pharmacophore is forced into 3 different conformations.

Further examples of RGD comprising peptide-based compounds are found in PCT/NO01/00146 and PCT/NO01/00390, the content of which are incorporated herein by reference.

The efficient targeting and imaging of integrin receptors associated with angiogenesis in vivo demands therefore a selective, high affinity RGD based vector that is chemically robust and stable. Furthermore, the route of excretion is an important factor when designing imaging agents in order to reduce problems with background. These stringent conditions are met by the bicyclic structures described in the present invention.

Description of the invention

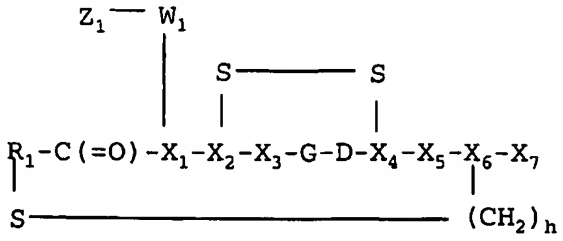
Viewed from one aspect the invention provides new peptide-based compounds of Formula I as defined in the claims. These compounds have affinity for integrin receptors, e.g. affinity for the integrin αvβ3.

The compounds of Formula I comprise at least two bridges, wherein one bridge forms a disulphide bond and the second bridge comprises a thioether (sulphide) bond and wherein the bridges fold the peptide moiety into a 'nested' configuration.

The compounds of the current invention thus have a maximum of one disulphide bridge per molecule moiety. Compounds defined by the present invention are surprisingly stable in vivo and under the conditions employed during labelling, e.g. during labelling with technetium.

These new compounds may be used in therapeutically effective treatments as well as for imaging purposes.

The new peptide-based compounds described in the present invention are defined by Formula I:



(I)

or physiologically acceptable salts thereof

wherein

G represents glycine, and

D represents aspartic acid, and

R_1 represents $-(CH_2)_n-$ or $-(CH_2)_n-C_6H_4-$, preferably R_1 represents $-(CH_2)-$, and

n represents a positive integer between 1 and 10, and

h represents a positive integer 1 or 2, and

X_1 represents an amino acid residue wherein said amino acid possesses a functional side-chain such as an acid or amine preferentially aspartic or glutamic acid, lysine, homolysine,diaminoalcylic acid or diaminopropionic acid,

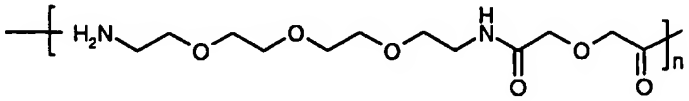
X_2 and X_4 represent independently an amino acid residue capable of forming a disulphide bond, preferably a cysteine or a homocysteine residue, and

X_3 represents arginine, N-methylarginine or an arginine mimetic, preferably an arginine, and

X_5 represents a hydrophobic amino acid or derivatives thereof, preferably a tyrosine, a phenylalanine, a 3-iodo-tyrosine or a naphthylalanine residue, and more preferably a phenylalanine or a 3-iodo-tyrosine residue, and

X_6 represents a thiol-containing amino acid residue, preferably a cysteine or a homocysteine residue, and

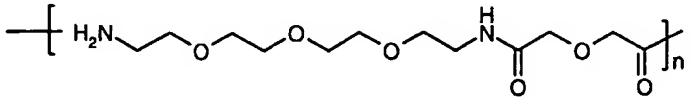
X_7 is absent or represents a homogeneous biomodifier moiety preferably based on a monodisperse PEG building block comprising 1 to 10 units of said building block, said biomodifier having the function of modifying the pharmacokinetics and blood clearance rates of the said agents. In addition X_7 may also represent 1 to 10 amino acid residues preferably glycine, lysine, aspartic acid or serine. In a preferred embodiment of this invention X_7 represents a biomodifier unit comprised of polymerisation of the monodisperse PEG-like structure, 17-amino-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid of Formula II,



(II)

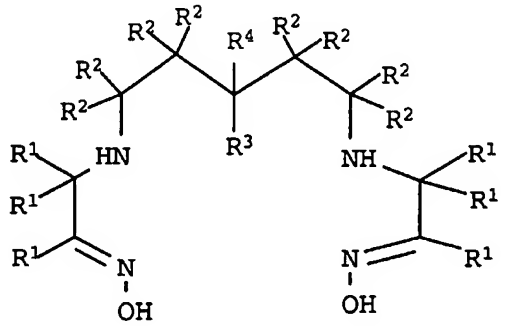
wherein n equals an integer from 1 to 10 and where the C-terminal unit is an amide moiety.

W_1 is absent or represents a spacer moiety and is preferentially derived from glutaric and/or succinic acid and/or a polyethyleneglycol based unit and/or a unit of Formula II



(II)

Z_1 is an antineoplastic agent, a chelating agent or a reporter moiety that can be represented by a chelating agent of Formula III



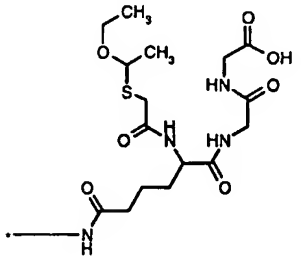
(III)

where:

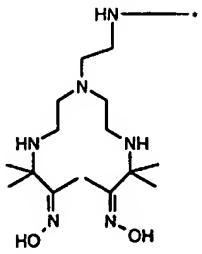
each R^1, R^2, R^3 and R^4 is independently an R group;

each R group is independently H or C_{1-10} alkyl, C_{3-10} alkylaryl, C_{2-10} alkoxyalkyl, C_{1-10} hydroxyalkyl, C_{1-10} alkylamine, C_{1-10} fluoroalkyl, or 2 or more R groups, together with the atoms

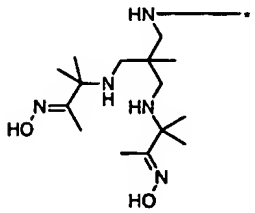
to which they are attached form a carbocyclic, heterocyclic, saturated or unsaturated ring,
or can represent a chelating agent given by formulas a, b, c and d.



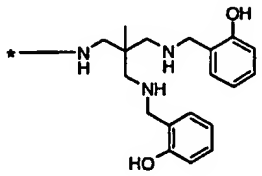
a



b

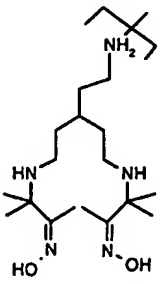


c



d

A preferred example of a chelating agent is represented by formula e.



e

Conjugates comprising chelating agents of Formula III can be radiolabelled to give good radiochemical purity, RCP, at room temperature, under aqueous conditions at near neutral pH. The risk of opening the disulphide bridges of the peptide component at room temperature is less than at an elevated temperature. A further advantage of radiolabelling the conjugates at room temperature is a simplified procedure in a hospital pharmacy.

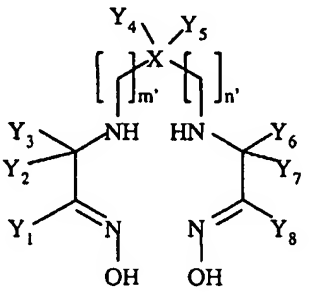
The role of the spacer moiety W_1 is to distance the relatively bulky chelating agent from the active site of the peptide component. The spacer moiety W_1 is also applicable to distance a bulky antineoplastic agent from the active site of the peptide.

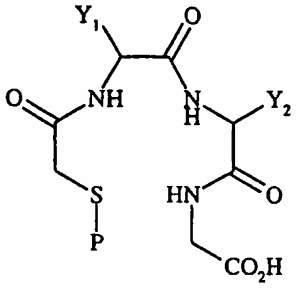
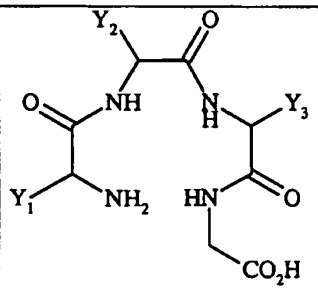
It is found that the biomodifier, X_7, modifies the pharmacokinetics and blood clearance rates of the compounds. The biomodifier effects less uptake of the compounds in tissue i.e. muscle, liver etc. thus giveing a better diagnostic image due to less background interference. The secretion is mainly through the kidneys due to a further advantage of the biomodifier.

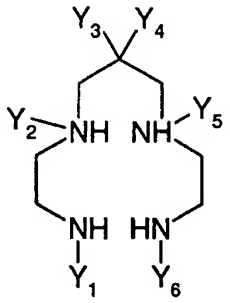
However the compounds defined in Formula I may also comprise chelating agents, Z1, as defined in Table I.

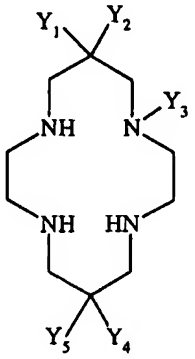
In some aspects of the invention, Z_1 comprises a reporter moiety where said reporter moiety comprises a radionuclide. Further definisions of chelating agents are listed in the following Table I.

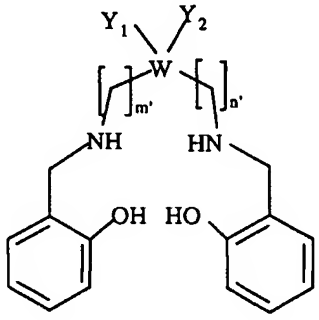
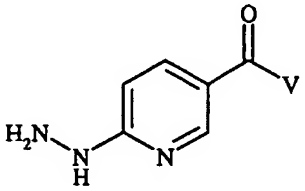
Table I:

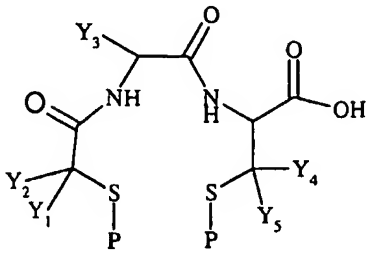
Class of ligand	Structure	Definitions
Amineoxime		Y 1-8 can be H, alkyl, aryl or combinations thereof and Y4 or Y5 contains a suitable functionality such that it can be conjugated to the peptide vector - e.g. preferably alkylamine, alkylsulphide, alkoxy, alkyl carboxylate, arylamine, aryl sulphide or α-haloacetyl X= C or N when m'=n'= 1 X= N when m'=n'= 2

Class of ligand	Structure	Definitions
MAG3 type		P = protecting group (preferably. benzoyl, acetyl, EOE); Y1, Y2 contains a suitable functionality such that it can be conjugated to the peptide vector; preferably H (MAG3), or the side chain of any amino acid, in either L or D form.
G4 type ligands		Y1, Y2, Y3 - contains a suitable functionality such that it can be conjugated to the peptide vector; preferably H, or the side chain of any amino acid, in either L or D form.

Class of ligand	Structure	Definitions
Tetra-amine ligands		Y1-Y6 can be H, alkyl, aryl or combinations thereof where the Y1-6 groups contain one or more functional moieties such that the chelate can be conjugated to the vector - e.g. preferably alkylamine, alkylsulphide, alkoxy, alkyl carboxylate, arylamine, aryl sulphide or α-haloacetyl

Class of ligand	Structure	Definitions
Cylam type ligands		Y1-5 can be H, alkyl, aryl or combinations thereof and where Y1-5 groups contain one or more functional moieties such that the chelate can be conjugated to the vector - e.g. preferably alkylamine, alkylsulphide, alkoxy, alkyl carboxylate, arylamine, aryl sulphide or α-haloacetyl

Class of ligand	Structure	Definitions
Diaminedi phenol		Y1, Y2 - H, alkyl, aryl and where Y1 or Y2 groups contains a functional moiety such that the chelate can be conjugated to the vector - e.g. preferably alkylamine, alkylsulphide, alkoxy, alkyl carboxylate, arylamine, aryl sulphide or α-haloacetyl W= C, N m'=n' = 1 or 2
HYNIC		V= linker to vector or vector itself.

Class of ligand	Structure	Definitions
Amide thiols		P = protecting group (preferably. benzoyl, acetyl, EOE); Y 1-5 = H, alkyl, aryl; or Y3 is a L or D amino acid side-chain or glycine.and the carboxylate may be used for conjugation to the vector via an amide bond. Alternatively the R_{1-5} groups may contain additional functionality such that the chelate can be conjugated to the vector - e.g. alkylamine, alkylsulphide, alkoxy, alkyl carboxylate, arylamine, aryl sulphide or α-haloacetyl.

In some aspects of the invention of Formula I the Z_1 moiety comprises the binding of a ^{18}F isotope or an isotope of Cu, incorporation into the agent either as a prosthetic group or by substitution or addition reactions. The resulting compound may thus be used in Positron Emission Tomography (PET) Imaging.

In one aspect of the present invention of formula I Z_1 is represented by an antineoplastic agent. In this aspect the compound will target an angiogenic site associated with cancer and bring the antineoplastic agent to the diseased area.

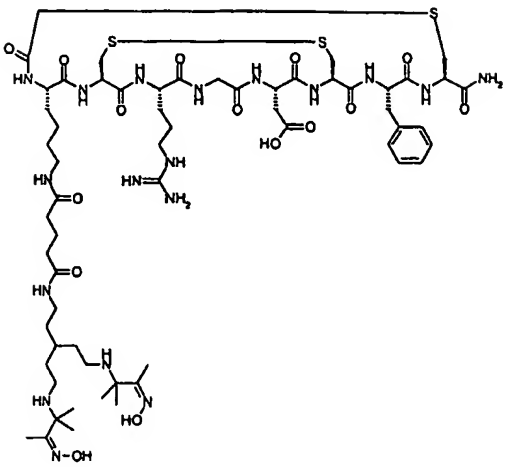
The antineoplastic agent may be represented by cyclophosphamide, chloroambucil, busulphan, methotrexate, cytarabine, fluorouracil, vinblastine, paclitaxel, doxorubicin, daunorubicin, etoposide, teniposide, cisplatin, amsacrine, docetaxel, but a wide range of other antineoplastic agents may also be used.

The peptide component of the conjugates described herein have preferably no free amino- or carboxy-termini. This introduces into these compounds a significant increase in resistance against enzymatic degradation and as a result they have an increased in vivo stability as compared to many known free peptides.

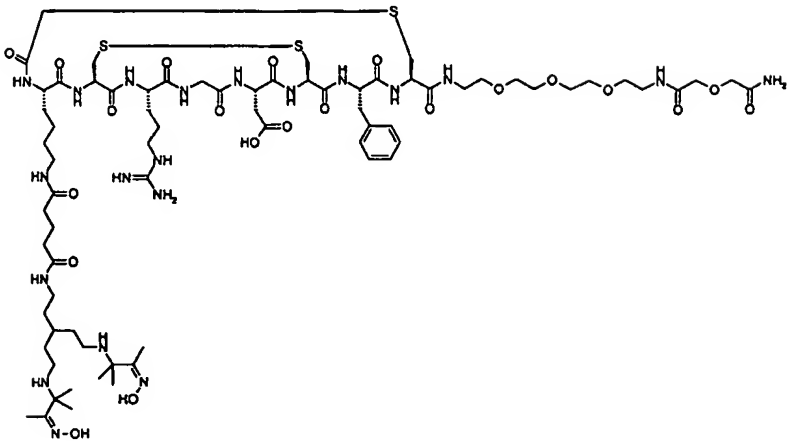
As used herein the term 'amino acid' refers in its broadest sense to proteogenic L-amino acids, D-amino acids, chemically modified amino acids, N-methyl, Cα-methyl and amino acid side-chain mimetics and unnatural amino acids such as naphthylalanine. Any naturally occurring amino acid or mimetics of such natural occurring amino acids are preferred.

Some preferred embodiments of the compounds of formula I are illustrated by compounds I-IV below:

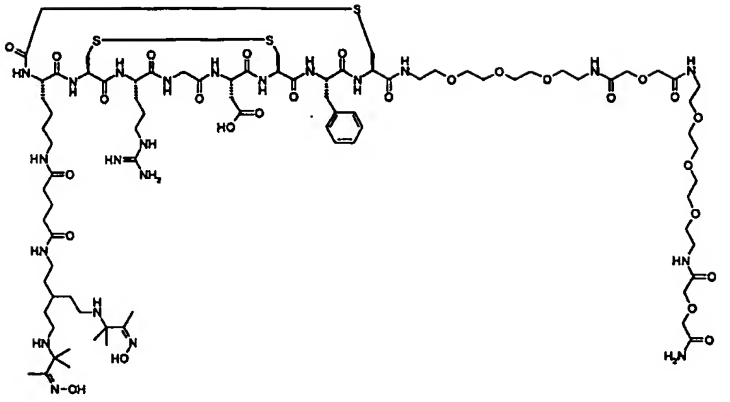
Compound I



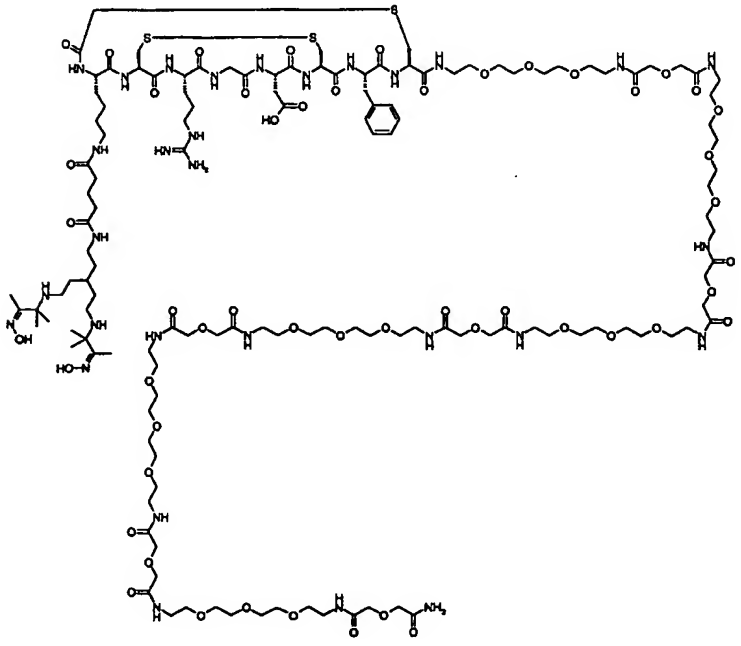
Compound II



Compound III



Compound IV



In most cases, it is preferred that the amino acids in the peptide are all in the L-form. However, in some embodiments of the invention one, two, three or more of the amino acids in the peptide are preferably in the D-form. The inclusion of such D-form amino acids can have a significant effect on the serum stability of the compound.
According to the present invention, any of the amino acid residues as defined in formula I may preferably represent a naturally occurring amino acid and independently in any of the D or L conformations.

Some of the compounds of the invention are high affinity RGD based vectors. As used herein the term 'high affinity RGD based vector' refers to compounds that have a Ki of < 10 nM and preferably < 5 nM, in a competitive binding assay for αvβ3 integrin and where the Ki value was determined by competition with the known high affinity ligand echistatin. Methods for carrying out such competition assays are well known in the art.

The present invention also provides a pharmaceutical composition comprising an effective amount (e.g. an amount effective for enhancing image contrast in in vivo imaging) of a compound of general formula I or a salt thereof, together with one or more pharmaceutically acceptable adjuvants, excipients or diluents.

The invention further provides a pharmaceutical composition for treatment of a disease comprising an effective amount of a compound of general formula I, or an acid addition salt thereof, together with one or more pharmaceutically acceptable adjuvants, excipients or diluents.

Other representative spacer (W_1) elements include structural-type polysaccharides, storage-type polysaccharides, polyamino acids and methyl and ethyl esters thereof, and polypeptides, oligosaccharides and oligonucleotides, which may or may not contain enzyme cleavage sites.

The reporter moieties (Z_1) in the contrast agents of the invention may be any moiety capable of detection either directly or indirectly in an in vivo diagnostic imaging procedure. Preferably the contrast agent comprises one reporter. Preferred are moieties which emit or may be caused to emit detectable radiation (e.g. by radioactive decay).

For MR imaging the reporter will either be a non zero nuclear spin isotope (such as ^{19}F) or a material having unpaired electron spins and hence paramagnetic, superparamagnetic, ferrimagnetic or ferromagnetic properties; for light imaging the reporter will be a light scatterer (e.g. a coloured or uncoloured particle), a light absorber or a light emitter; for magnetometric imaging the reporter will have detectable magnetic properties; for electrical impedance imaging the reporter will affect electrical impedance; and for scintigraphy, SPECT, PET, and the like, the reporter will be a radionuclide.

Stated generally, the reporter may be (1) a chelatable metal or polyatomic metal-containing ion (i.e. TcO, etc), where the metal is a high atomic number metal (e.g. atomic number greater than 37), a paramagentic species (e.g. a transition metal or lanthanide), or a radioactive isotope, (2) a covalently bound non-metal species which is an unpaired electron site (e.g. an oxygen or carbon in a persistant free

radical), a high atomic number non-metal, or a radioisotope, (3) a polyatomic cluster or crystal containing high atomic number atoms, displaying cooperative magnetic behaviour (e.g. superparamagnetism, ferrimagnetism or ferromagnetism) or containing radionuclides.

Examples of particular preferred reporter groups (Z_1) are described in more detail below.

Chelated metal reporters are preferably chosen from the group below; ^{90}Y, ^{99m}Tc, ^{111}In, ^{47}Sc, ^{67}Ga, ^{51}Cr, ^{177m}Sn, ^{67}Cu, ^{167}Tm, ^{97}Ru, ^{188}Re, ^{177}Lu, ^{199}Au, ^{203}Pb and ^{141}Ce.

The metal ions are desirably chelated by chelant groups on the linker moiety. Further examples of suitable chelant groups are disclosed in US-A-4647447, WO89/00557, US-A-5367080, US-A-5364613.

Methods for metallating any chelating agents present are within the level of skill in the art. Metals can be incorporated into a chelant moiety by any one of three general methods: direct incorporation, template synthesis and/or transmetallation. Direct incorporation is preferred.

Thus it is desirable that the metal ion be easily complexed to the chelating agent, for example, by merely exposing or mixing an aqueous solution of the chelating agent-containing moiety with a metal salt in an aqueous solution preferably having a pH in the range of about 4 to about 11. The salt can be any salt, but preferably the salt is a water soluble salt of the metal such as a halogen salt, and more preferably such salts are selected so as not to interfere with the binding of the metal ion with the chelating agent.

The chelating agent-containing moiety is preferrably in aqueous solution at a pH of between about 5 and about 9, more preferably between pH about 6 to about 8. The chelating agent-containing moiety can be mixed with buffer salts such as citrate, carbonate, acetate, phosphate and borate to produce the optimum pH. Preferably, the buffer salts are selected so as not to interfere with the subsequent binding of the metal ion to the chelating agent.

The following isotopes or isotope pairs can be used for both imaging and therapy without having to change the radiolabeling methodology or chelator: $^{47}Sc_{21}$; $^{141}Ce_{58}$; $^{188}Re_{75}$; $^{177}Lu_{71}$; $^{199}Au_{79}$; $^{47}Sc_{21}$; $^{131}I_{53}$; $^{67}Cu_{29}$; $^{131}I_{53}$ and $^{123}I_{53}$; $^{188}Re_{75}$ and $^{99m}Tc_{43}$; $^{90}Y_{39}$ and $^{87}Y_{39}$; $^{47}Sc_{21}$ and $^{44}Sc_{21}$; $^{90}Y_{39}$ and $^{123}I_{53}$; $^{146}Sm_{62}$ and $^{153}Sm_{62}$; and $^{90}Y_{39}$ and $^{111}In_{49}$.

Preferred non-metal atomic reporters include radioisotopes such as ^{123}I, ^{131}I and ^{18}F as well as non zero nuclear spin atoms such as ^{19}F, and heavy atoms such as I.

In a further embodiment of this invention, the use of radioisotopes of iodine or fluorine is specifically contemplated. For example, if the peptide or linker is comprised of substituents that can be chemically substituted by iodine or fluorine in a covalent bond forming reaction, such as, for example, substituents containing hydroxyphenyl or p-nitrobenzoyl functionality, such substituents can be labeled by methods well known in the art with a radioisotope of iodine or fluorine respectively. These species can be used in therapeutic and diagnostic imaging applications. While, at the same time, a metal attached to a chelating agent on the same peptide-linker can also be used in either therapeutic or diagnostic imaging applications.

A preferred embodiment of the invention relates to a radiolabelled agent of general formula (I), particularly for use in tumour imaging.

The diagnostic agents of the invention may be administered to patients for imaging in amounts sufficient to yield the desired contrast with the particular imaging technique. Where the reporter is a metal, generally dosages of from 0.001 to 5.0 mmoles of chelated imaging metal ion per kilogram of patient bodyweight are effective to achieve adequate contrast enhancements. Where the reporter is a radionuclide, dosages of 0.01 to 100 mCi, preferably 0.1 to 50 mCi will normally be sufficient per 70kg bodyweight.

The dosage of the compounds of the invention for therapeutic use will depend upon the condition being treated, but in general will be of the order of from 1 pmol/kg to 1 mmol/kg bodyweight.

The compounds according to the invention may therefore be formulated for administration using physiologically acceptable carriers or excipients in a manner fully within the skill of the art. For example, the compounds, optionally with the addition of pharmaceutically acceptable excipients, may be suspended or dissolved in an aqueous medium, with the resulting solution or suspension then being sterilized.

The compounds of formula I may be therapeutically effective in the treatment of disease states as well as detectable in in vivo imaging. Thus for example the vector on the reporter moieites may have therapeutic efficacy, e.g. by

virtue of the radiotherapeutic effect of a radionuclide reporter of the vector moiety.

Use of the compounds of formula I in the manufacture of therapeutic compositions (medicament) and in methods of therapeutic or prophylactic treatment, preferably treatment of cancer, of the human or animal body are thus considered to represent further aspects of the invention.

Further examples of the reporters which may be used in the context of the current application are given on pages 63-66 and 70-86 of WO98/47541 and the disclosures made on these pages are incorporated herein by reference in their entirety. It is hereby asserted that each and every reporter or part thereof disclosed on the aforementioned pages is considered to be part of the description of the invention contained in this application.

Viewed from a further aspect the invention provides the use of a compound of formula I for the manufacture of a contrast medium for use in a method of diagnosis involving administration of said contrast medium to a human or animal body and generation of an image of at least part of said body.

Viewed from a still further aspect the invention provides a method of generating an image of a human or animal body involving administering a contrast agent to said body, e.g. into the vascular system and generating an image of at least a part of said body to which said contrast agent has distributed using scintigraphy, PET or SPECT modalities, wherein as said contrast agent is used an agent of formula I.

Viewed from a still further aspect the invention provides a method of generating enhanced images of a human or animal body previously administered with a contrast agent composition comprising a compound as defined by formula I, which method comprises generating an image of at least part of said body.

Viewed from a further aspect the invention provides a method of monitoring the effect of treatment of a human or animal body with a drug to combat a condition associated with cancer, preferably angiogenesis, e.g. a cytotoxic agent, said method involving administering to said body an agent of formula I and detecting the uptake of said agent by cell receptors, preferably endothelial cell receptors and in particular αvβ3 receptors, said administration and detection optionally but preferably being effected repeatedly, e.g. before, during and after treatment with said drug.

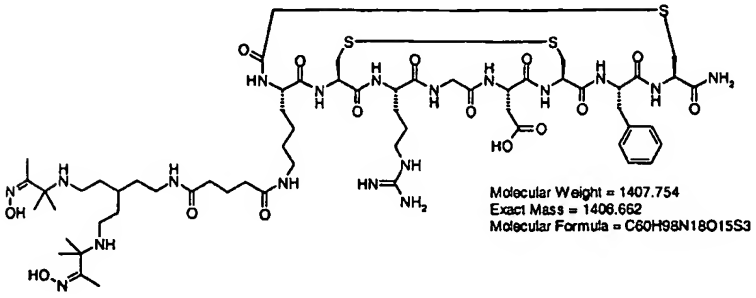
The compounds of the present invention can be synthesised using all the known methods of chemical synthesis but particularly useful is the solid-phase methodology of Merrifield employing an automated peptide synthesiser (J. Am. Chem. Soc., 85: 2149 (1964)). The peptides and peptide chelates may be purified using high performance liquid chromatography (HPLC) and characterised by mass spectrometry and analytical HPLC before testing in the in vitro screen.

The present invention will now be further illustrated by way of the following non-limiting examples.

Examples:

Example 1:

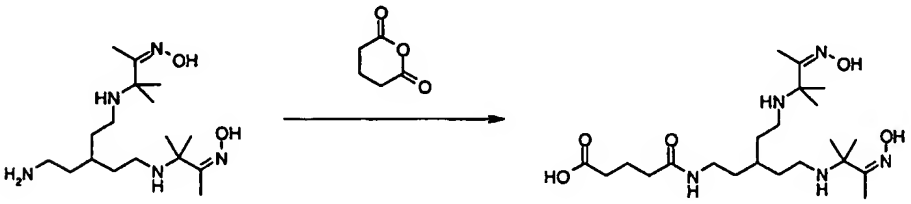
Synthesis of disulfide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-NH_2



1 a) Synthesis of cPn216 chelate

For details of the synthesis of technetium chelate cPn216 the reader is referred to patent filing GB0116815.2

1 b) Synthesis of cPn216-glutaric acid intermediate

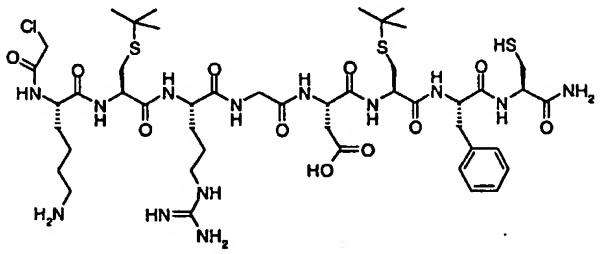


cPn216 (100 mg, 0.29 mmol) was dissolved in DMF (10 mL) and glutaric anhydride (33 mg, 0.29 mmol) added by portions with stirring. The reaction was stirred for 23 hours to afford complete conversion to the desired product. The pure acid was obtained following RP-HPLC in good yield.

1 c) Synthesis of tetrafluorothiophenyl ester of cPn216-glutaric acid

To cPn216-glutaric acid (300 mg, 0.66 mmol) in DMF (2 mL) was added HATU (249 mg, 0.66 mmol) and NMM (132 µL, 1.32 mmol). The mixture was stirred for 5 minutes then tetrafluorothiophenol (0.66 mmol, 119 mg) was added. The solution was stirred for 10 minutes then the reaction mixture was diluted with 20 % acetonitrile/water (8 mL) and the product purified by RP-HPLC yielding 110 mg of the desired product following freeze-drying.

1 d) Synthesis of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-NH_2



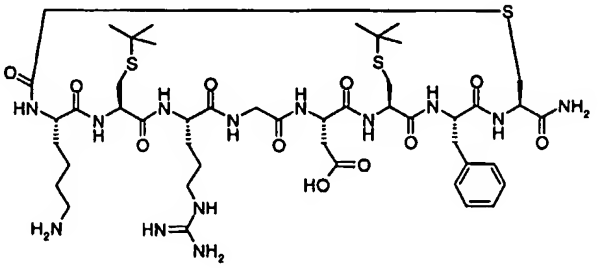
Molecular Weight = 1118.844
Exact Mass = 1117.464
Molecular Formula = C46H76ClN13O11S3

The peptide was synthesised on an ABI 433A automatic peptide synthesiser starting with Rink Amide AM resin on a 0.25 mmol scale using 1 mmol amino acid cartridges. The amino acids were pre-activated using HBTU before coupling. N-terminal amine groups were chloroacetylated using a solution of chloroacetic anhydride in DMF for 30 min.

The simultaneous removal of peptide and side-chain protecting groups (except tBu) from the resin was carried out in TFA containing TIS (5 %), H_20 (5 %) and phenol (2.5 %) for two hours.

After work-up 295 mg of crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.42 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1118.5, found, at 1118.6).

1 e) Synthesis of thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-NH_2



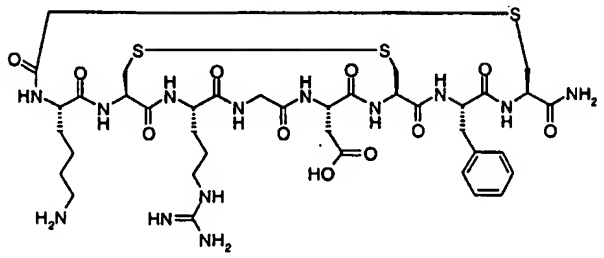
Molecular Weight = 1082.383
Exact Mass = 1081.487
Molecular Formula = C46H75N13O11S3

295 mg of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-NH_2 was dissolved in water/acetonitrile. The mixture was adjusted to pH 8 with ammonia solution and stirred for 16 hours.

After work-up 217 mg of crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.18 min). Further product

characterisation was carried out using mass spectrometry: Expected, M+H at 1882.5, found, at 1882.6).

1 f) Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-NH_2

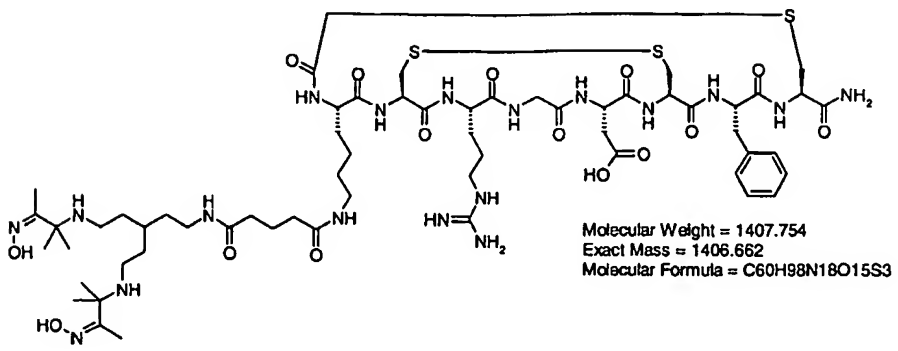


Molecular Weight = 968.150
Exact Mass = 967.346
Molecular Formula = C38H57N13O11S3

217 mg of thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-NH_2 was treated with a solution of anisole (500 µL), DMSO (2 mL) and TFA (100 mL) for 60 min following which the TFA was removed in vacuo and the peptide precipitated by the addition of diethyl ether.

Purification by preparative HPLC (Phenomenex Luna 10 µ C18 (2) 250 x 50 mm column) of the crude material (202 mg) was carried out using 0-30 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 60 min at a flow rate of 50 mL/min. After lyophilisation 112 mg of pure material was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 5.50 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 968, found, at 971).

1 g) Synthesis of disulfide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-NH_2



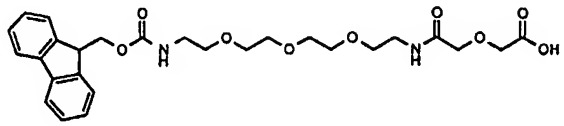
9.7 mg of disulphide[Cys^{2-6}] thioether cyclo[CH_2CO-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys]-NH_2, 9.1 mg of cPn216 chelate active ester and 6 µL of N-methylmorpholine was dissolved in DMF (0.5 mL). The mixture was stirred for 3 hours.

Purification by preparative HPLC (Phenomenex Luna 5 µ C18 (2) 250 x 21.20 mm column) of the reaction mixture was carried out using 0-30 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 5.7 mg of pure material was obtained (Analytical HPLC: Gradient, 0-30 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 7.32 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1407.7, found, at 1407.6).

Example 2:

Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 1.

2 a) Synthesis of 17-(Fmoc-amino)-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid



This building block is coupled to the solid-phase using Fmoc chemistry. The coupled form of this building block will be referred to in short as $(PEG)_n$ where n is a positive integer.

1,11-Diazido-3,6,9-trioxaundecane

A solution of dry tetraethylene glycol (19.4 g, 0.100 mol) and methanesulphonyl chloride (25.2 g, 0.220 mol) in dry THF (100 ml) was kept under argon and cooled to 0 °C in an ice/water bath. To the flask was added a solution of triethylamine (22.6 g, 0.220 mol) in dry THF (25 ml) dropwise over 45 min. After 1 hr the cooling bath was removed and stirring was continued for 4 hrs. Water (60 ml) was added. To the mixture was added sodium hydrogencarbonate (6 g, to pH 8) and sodium azide (14.3 g, 0.220 mmol), in that order. THF was removed by distillation and the aqueous solution was refluxed for 24 h (two layers formed). The mixture was cooled and ether (100 ml) was added. The aqueous phase was saturated with sodium chloride. The phases were separated and the aqueous phase was extracted with ether (4 x 50 ml). Combined organic phases were washed with brine (2 x 50 ml) and dried ($MgSO_4$). Filtration and concentration gave 22.1 g (91%) of yellow oil. The product was used in the next step without further purification.

11-Azido-3,6,9-trioxaundecanamine

To a mechanically, vigorously stirred suspension of 1,11-diazido-3,6,9-trioxaundecane (20.8 g, 0.085 mol) in 5% hydrochloric acid (200 ml) was added a solution of triphenylphosphine (19.9 g, 0.073 mol) in ether (150 ml) over 3 hrs at room temperature. The reaction mixture was stirred for additional 24 hrs. The phases were separated and the aqueous phase was extracted with dichloromethane (3 x 40 ml). The aqueous phase was cooled in an ice/water bath and pH was adjusted to ca 12 by addition of KOH. The product was extracted into dichloromethane (5 x 50 ml). Combined organic phases were dried ($MgSO_4$). Filtration and evaporation gave 14.0 g (88%) of yellow oil. Analysis by MALDI-TOF mass spectroscopy (matrix: α-cyano-4-hydroxycinnamic acid) gave a M+H peak at 219 as expected. Further characterisation using ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopy verified the structure.

17-Azido-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid

To a solution of 11-azido-3,6,9-trioxaundecanamine (10.9 g, 50.0 mmol) in dichloromethane (100 ml) was added diglycolic anhydride (6.38 g, 55.0 mmol). The reaction mixture was stirred overnight. HPLC analysis (column Vydac 218TP54; solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 4-16% B over 20 min; flow 1.0 ml/min; UV detection at 214 and 284 nm), showed complete conversion of starting material to a product with retention time 18.3 min. The solution was concentrated to give quantitative yield of a yellow syrup. The product was analysed by LC-MS (ES ionisation) giving [MH]+ at 335 as expected. ^1H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopy was in agreement with structure

The product was used in the next step without further purification.

17-Amino-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid

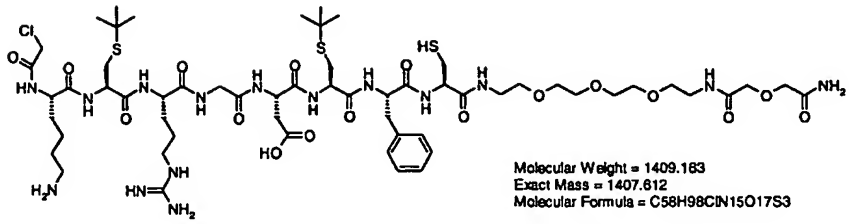
A solution of 17-azido-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid (8.36 g, 25.0 mmol) in water (100 ml) was reduced using H_2(g)-Pd/C (10%). The reaction was run until LC-MS analysis showed complete conversion of starting material (column Vydac 218TP54; solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 4-16% B over 20 min; flow 1.0 ml/min; UV detection at 214 and 284 nm, ES ionisation giving M+H at 335 for starting material and 309 for the product). The solution was filtered and used directly in the next step.

17-(Fmoc-amino)-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid

To the aqueous solution of 17-amino-5-oxo-6-aza-3,9,12,15-tetraoxaheptadecanoic acid from above(corresponding to 25.0 mmol amino acid) was added sodium bicarbonate (5.04 g, 60.0 mmol) and dioxan (40 ml). A solution of Fmoc-chloride (7.11 g, 0.275 mol) in dioxan (40 ml) was added dropwise. The reaction mixture was stirred overnight. Dioxan was evaporated off (rotavapor) and the aqueous phase was extracted with ethyl acetate. The aqueous phase was acidified by addition of hydrochloric acid and precipitated material was extracted into chloroform. The organic phase was dried ($MgSO_4$), filtered and concentrated to give 11.3 g (85%) of a yellow syrup. The structure was confirmed by LC-MS analysis (column Vydac 218TP54; solvents: A = water/0.1% TFA and B = acetonitrile/0.1% TFA; gradient 40-60% B over 20 min; flow 1.0 ml/min; UV detection at 214 and 254 nm, ES ionisation

giving M+H at 531 as expected for the product peak at 5,8 minutes). The analysis showed very low content of side products and the material was used without further purification.

2 b) Synthesis of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-(PEG)n-NH_2 where n= 1

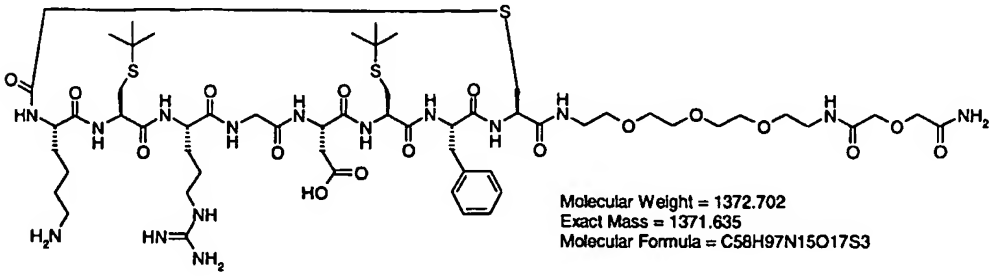


The PEG unit was coupled manually to Rink Amide AM resin, starting on a 0.25 mmol scale, mediated by HATU activation. The remaining peptide was assembled on an ABI 433A automatic peptide synthesiser using 1 mmol amino acid cartridges. The amino acids were pre-activated using HBTU before coupling. N-terminal amine groups were chloroacetylated using a solution of chloroacetic anhydride in DMF for 30 min.

The simultaneous removal of peptide and side-chain protecting groups (except tBu) from the resin was carried out in TFA containing TIS (5 %), H_20 (5 %) and phenol (2.5 %) for two hours.

After work-up 322 mg of crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.37 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1409, found, at 1415).

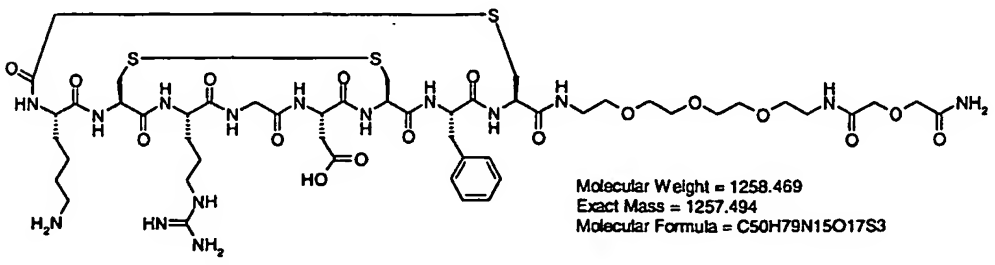
2 c) Synthesis of thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-(PEG)n-NH_2 where n= 1



322 mg of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-(PEG)n-NH_2 was dissolved in water/acetonitrile. The mixture was adjusted to pH 8 with ammonia solution and stirred for 16 hours.

After work-up crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.22 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1373, found, at 1378).

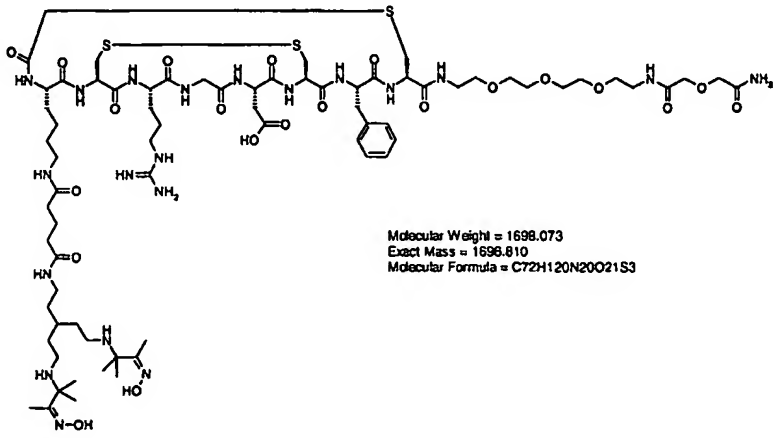
2 d) Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 1



Thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-(PEG)n-NH_2 was treated with a solution of anisole (200 µL), DMSO (2 mL) and TFA (100 mL) for 60 min following which the TFA was removed in vacuo and the peptide precipitated by the addition of diethyl ether.

Purification by preparative HPLC (Phenomenex Luna 5 µ C18 (2) 250 x 21.20 mm column) of 70 mg crude material was carried out using 0-30 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 46 mg of pure material was obtained (Analytical HPLC: Gradient, 0-30 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.80 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1258.5, found, at 1258.8).

2 e) Synthesis of disulfide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 1



13 mg of [Cys^{2-6}] cyclo[CH_2CO-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys]-(PEG)n-NH_2, 9.6 mg of cPn216 chelate active ester

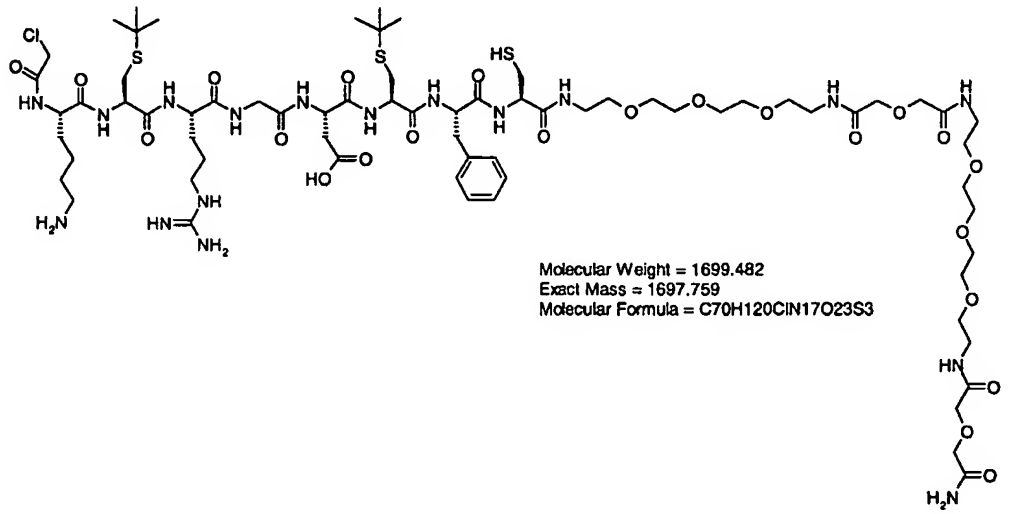
and 8 µL of N-methylmorpholine was dissolved in DMF (0.5 mL). The mixture was stirred for 2 hours and 30 minutes.

Purification by preparative HPLC (Phenomenex Luna 5 µ C18 (2) 250 x 21.20 mm column) of the reaction mixture was carried out using 0-30 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 14.2 mg of pure material was obtained (Analytical HPLC: Gradient, 0-30 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 7.87 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1697.8, found, at 1697.9).

Example 3:

Synthesis of disulfide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 2.

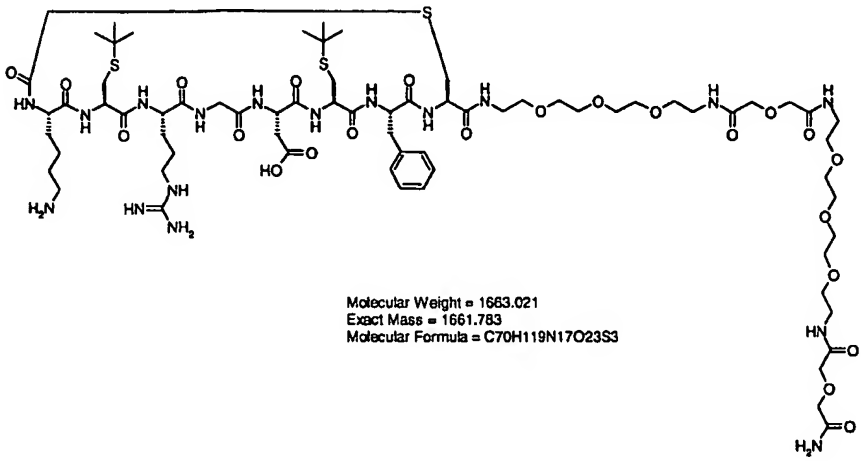
3 a) Synthesis of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-(PEG)n-NH_2 where n= 2



Assembly of peptide as for example 2 b), both PEG units coupled manually.

After work-up crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.40 min).

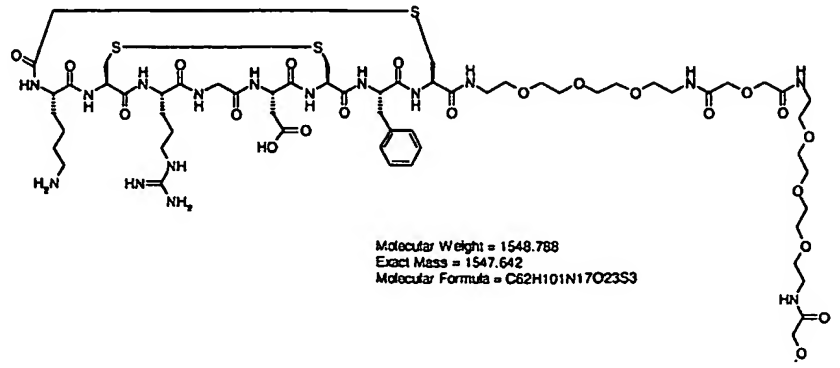
3 b) Synthesis of thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-(PEG)n-NH_2 where n=2



$ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-(PEG)n-NH_2 where n=2 was dissolved in water/acetonitrile. The mixture was adjusted to pH 8 with ammonia solution and stirred for 16 hours.

After work-up 380 mg of crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.28 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1663, found, at 1670).

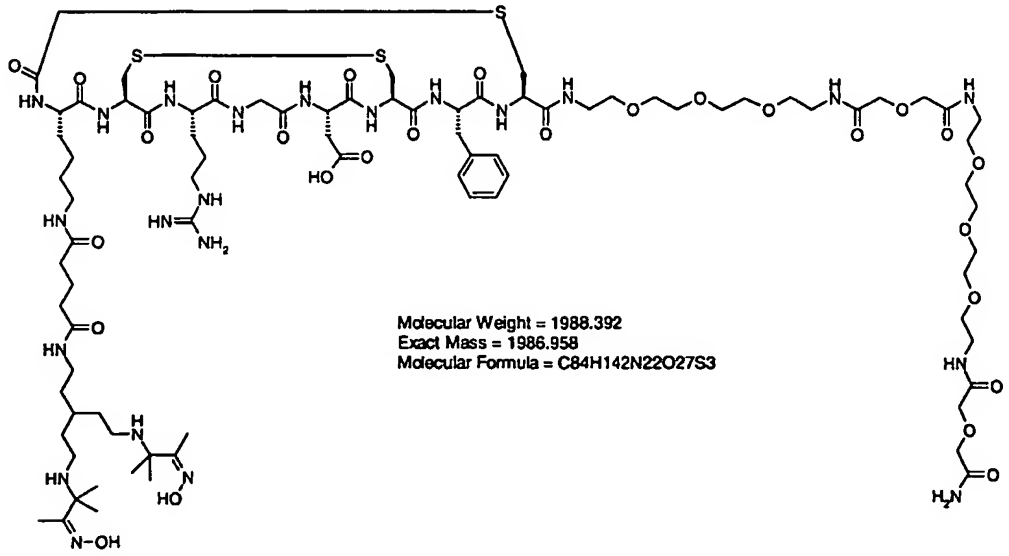
3 c) Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 2.



380 mg of thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-(PEG)n-NH_2 where n=2 was treated with a solution of anisole (500 µL), DMSO (2 mL) and TFA (100 mL) for 60 min following which the TFA was removed in vacuo and the peptide precipitated by the addition of diethyl ether.

Purification by preparative HPLC (Phenomenex Luna 10 µ C18 (2) 250 x 50 mm column) of the crude material (345 mg) was carried out using 0-30 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 60 min at a flow rate of 50 mL/min. After lyophilisation 146 mg of pure material was obtained (Analytical HPLC: Gradient, 0-30 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 7.42 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1548.6, found, at 1548.8).

3 d) Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 2.



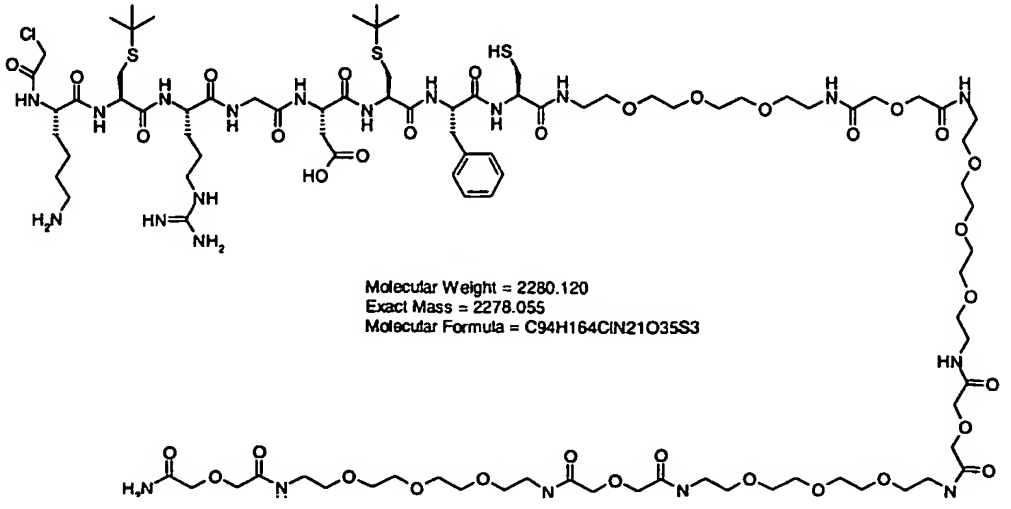
146 mg of [Cys^{2-6}] cyclo[CH_2CO-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys]-$(PEG)_2$-NH_2, 110 mg of cPn216 chelate active ester and 76 µL of N-methylmorpholine was dissolved in DMF (6 mL). The mixture was stirred for 9 hours.

Purification by preparative HPLC (Phenomenex Luna 10 µ C18 (2) 250 x 50 mm column) of the reaction mixture was carried out using 0-30 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 60 min at a flow rate of 50 mL/min. After lyophilisation 164 mg of pure material was obtained (Analytical HPLC: Gradient, 0-30 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 8.13 min). Further product characterisation was carried out using mass spectrometry: Expected, M+H at 1988.0, found, at 1988.0).

Example 4:

Synthesis of disulfide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 4.

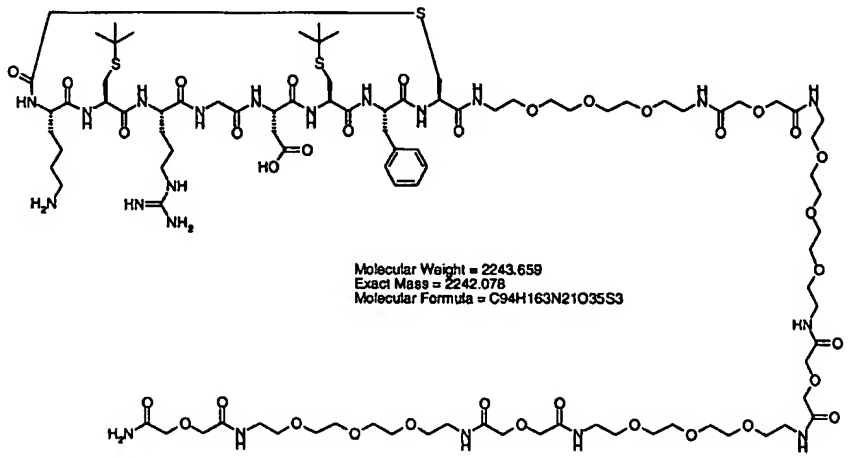
4 a) Synthesis of $ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-(PEG)n-NH_2 where n= 4



Assembly of peptide as for example 2 b), all four PEG units coupled manually.

After work-up crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.50 min).

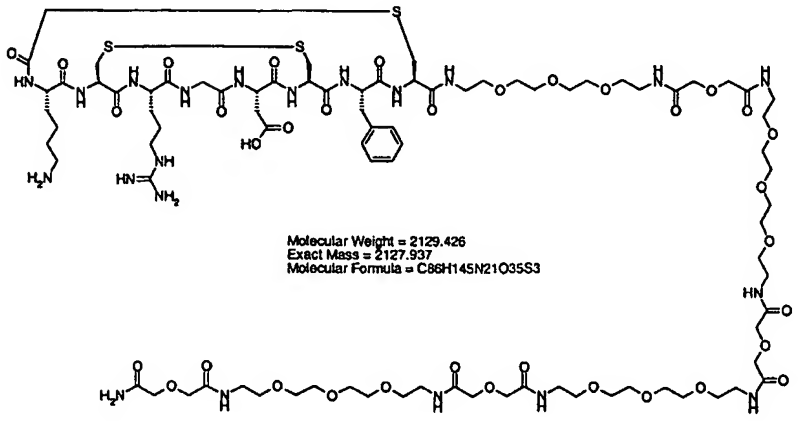
4 b) Synthesis of thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-(PEG)n-NH_2 where n= 4



$ClCH_2CO$-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys-$(PEG)_4$-NH_2 was dissolved in water/acetonitrile. The mixture was adjusted to pH 8 with ammonia solution and stirred for 16 hours.

After work-up crude peptide was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 6.37 min). Further product characterisation was carried out using mass spectrometry: Expected, [(M+2H)/2] at 1122.0, found, at 1122.5).

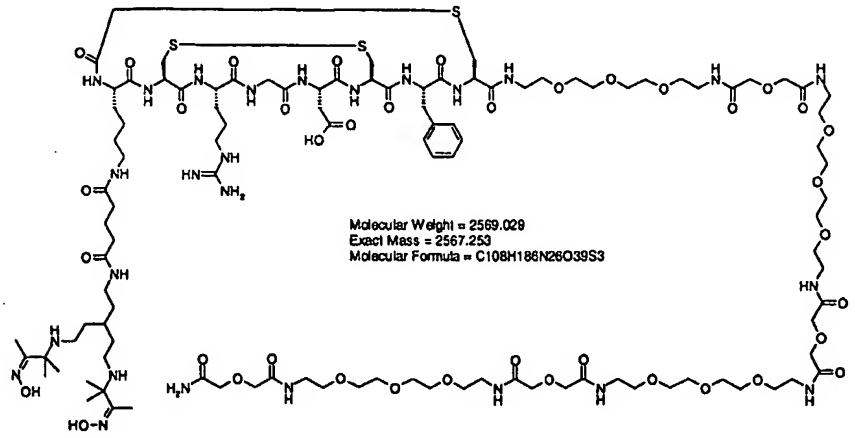
4 c) Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 4



Thioether cyclo[CH_2CO-Lys-Cys(tBu)-Arg-Gly-Asp-Cys(tBu)-Phe-Cys]-$(PEG)_4$-NH_2 was treated with a solution of anisole (100 µL), DMSO (1 mL) and TFA (50 mL) for 60 min following which the TFA was removed in vacuo and the peptide precipitated by the addition of diethyl ether.

Purification by preparative HPLC (Phenomenex Luna 5 µ C18 (2) 250 x 21.20 mm column) of the crude material (345 mg) was carried out using 5-50 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 12 mg of pure material was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 4.87 min).

4 d) Synthesis of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys(cPn216-glutaryl)-Cys^2-Arg-Gly-Asp-Cys^6-Phe-Cys]-(PEG)n-NH_2 where n= 4.



12 mg of disulphide [Cys^{2-6}] thioether cyclo[CH_2CO-Lys-Cys-Arg-Gly-Asp-Cys-Phe-Cys]-$(PEG)_4$-NH_2, 5.2 mg of cPn216 chelate active ester and 2 µL of N-methylmorpholine was dissolved in DMF (0.5 mL). The mixture was stirred for 7 hours.

Purification by preparative HPLC (Phenomenex Luna 5 µ C18 (2) 250 x 21.20 mm column) of the reaction mixture was carried out using 5-50 % B, where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA, over 40 min at a flow rate of 10 mL/min. After lyophilisation 8 mg of pure material was obtained (Analytical HPLC: Gradient, 5-50 % B over 10 min where A = H_2O/0.1 % TFA and B = CH_3CN/0.1 % TFA; column, Phenomenex Luna 3 µ C18 (2) 50 x 4.6 mm; flow, 2 mL/min; detection, UV 214 nm; product retention time, 5.17 min). Further product characterisation was carried out using mass spectrometry: Expected, [(M+2H)/2] at 1284.6, found, at 1284.9).

SEQUENCE LISTING

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<130> NO 20014954

<150> NO20014954

<151> 2001-10-11

<160> 1

<170> PatentIn version 3.1

<210> 1

<211> 8

<212> PRT

<213> Artificial sequence

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<223> Synthetic peptide

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<223> Disulphide bridge between amino acid residue 2 and 5.

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<223> Thioether bridge between amino acid residue 1 and 8.

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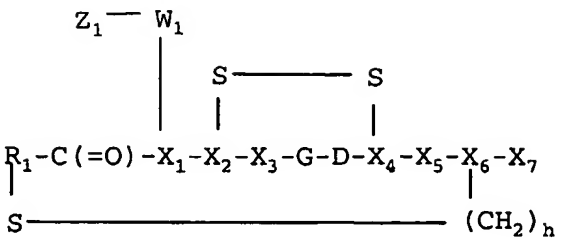
Lys Cys Arg Gly Asp Cys Phe Cys
1 5

Claims

1. A compound of general formula (I)



(I)

or pharmaceutically acceptable salt thereof
wherein

G represents glycine

D represents aspartic acid

R_1 represents $-(CH_2)_n-$ or $-(CH_2)_n-C_6H_4-$ wherein

n represents a positive integer 1 to 10

h represents a positive integer 1 or 2

X_1 represents an amino acid residue wherein said amino acid possesses a functional side-chain such as an acid or amine.

X_2 and X_4 represent independently an amino acid residue capable of forming a disulphide bond,

X_3 represents arginine, N-methylarginine or an arginine mimetic,

X_5 represents a hydrophobic amino acid or derivatives thereof, and

X_6 represents a thiol-containing amino acid residue, and

X_7 is absent or represents a biomodifier moiety

Z_1 represents an antineoplastic agent, a chelating agent or a reporter moiety and

W_1 is absent or represents a spacer moiety

2. A compound as claimed in claim 1 wherein any of the amino acid residues are independently in the D or L conformation.

3. A compound as claimed in claim 1 wherein R_1 represents -(CH_2)-.

4. A compound as claimed in any of claims 1 to 3 wherein X_1 represents aspartic acid, glutamic acid, lysine, homolysine or a diaminoalkylic acid or derivatives thereof.

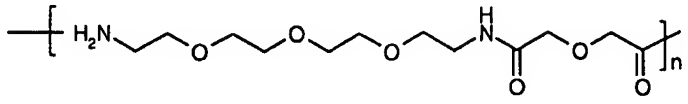
5. A compound as claimed in any of the previous claims wherein X_2, X_4 and X_6 independently represent a cysteine or homocysteine residue.

6. A compound as claimed in any of the previous claims wherein X_3 represents an arginine residue.

7. Compound as claimed in any of the previous claims wherein X_5 represents a tyrosine, a phenylalanine, a 3-iodo-tyrosine or a naphthylalanine residue.

8. A compound as claimed in any of the previous claims wherein X_7 is absent or comprises 1-10 units of a monodisperse PEG building block.

9. A compound as claimed in any of the previous claims wherein X_7 is absent or comprises 1-10 units of Formula II

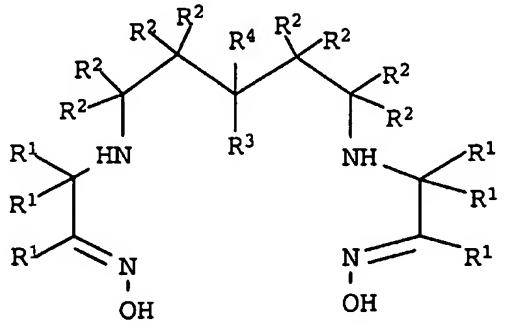


(II)

10. A compound as claimed in any of the previous claims wherein X_7 represent 1- 10 amino acid residues

11. A compound as claimed in any of the previous claims wherein X_7 represent glycine, lysine, aspartic acid or serine residues, preferably glycine.

12. A compound as claimed in any of the previous claims where Z_1 is a chelating agent of Formula III



(III)

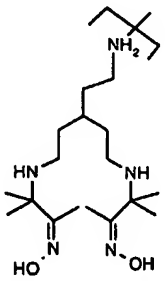
where:

each R^1, R^2, R^3 and R^4 is independently an R group;

each R group is independently H or C_{1-10} alkyl, C_{3-10} alkylaryl, C_{2-10} alkoxyalkyl, C_{1-10} hydroxyalkyl, C_{1-10} alkylamine, C_{1-10} fluoroalkyl, or 2 or more R groups, together with the atoms

to which they are attached form a carbocyclic, heterocyclic, saturated or unsaturated ring.

13. A compound as claimed in any of the previous claims where Z_1 is



e

14. A compound as claimed in any of the previous claims wherein Z_1 comprises a reporter moiety.

15. A compound as claimed in claim 14 wherein the reporter moiety comprises metal radionuclides, paramagnetic metal ions, fluorescent metal ions, heavy metal ions or cluster ions.

16. A compound as claimed in claims 14 and 15 wherein the reporter moiety comprises ^{90}Y, ^{99m}Tc, ^{111}In, ^{47}Sc, ^{67}Ga, ^{51}Cr, ^{177m}Sn, ^{67}Cu, ^{167}Tm, ^{97}Ru, ^{188}Re, ^{177}Lu, ^{199}Au, ^{203}Pb, ^{141}Ce or ^{18}F.

17. A compound as claimed in claims 1-16 wherein the reporter moiety is ^{99m}Tc.

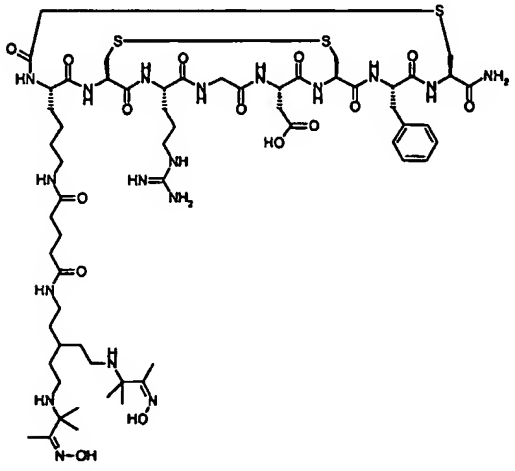
18. A compound as claimed in claims 1-11 where Z_1 is an antineoplastic agent.

19. A compound as claimed in claim 18 where Z_1 represent cyclophosphamide, chloroambucil, busulphan, methotrexate, cytarabine, fluorouracil, vinblastine, paclitaxel, doxorubicin, daunorubicin, etoposide, teniposide, cisplatin, amsacrine or docetaxel.

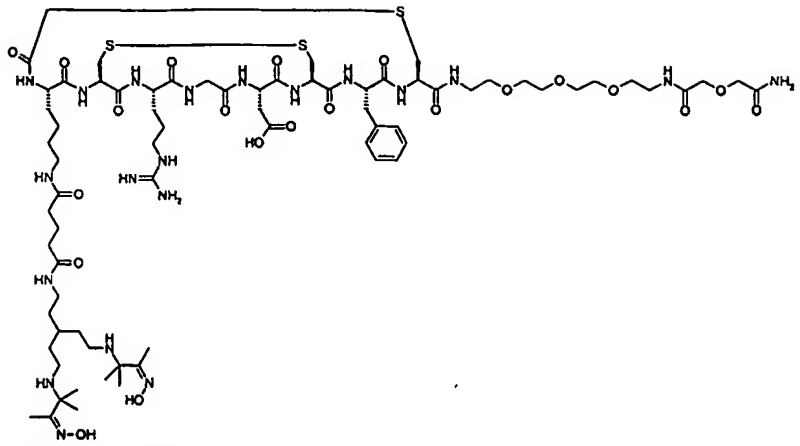
20. A compound as claimed in any of the previous claims where W_1 is glutaric or succinic acid

21. A compound as claimed in claim 1 defined by the following formulas

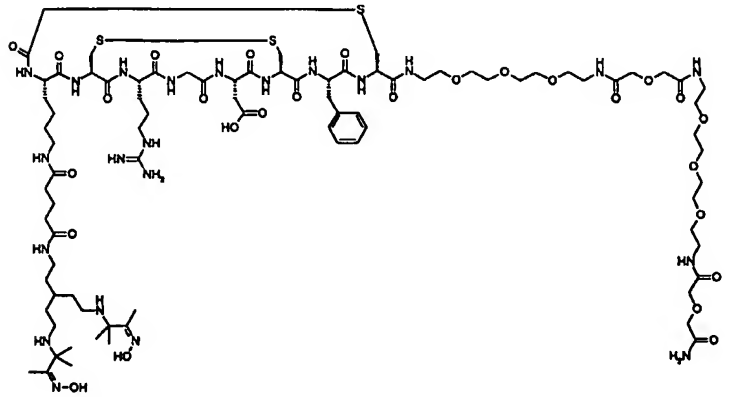
Compound I



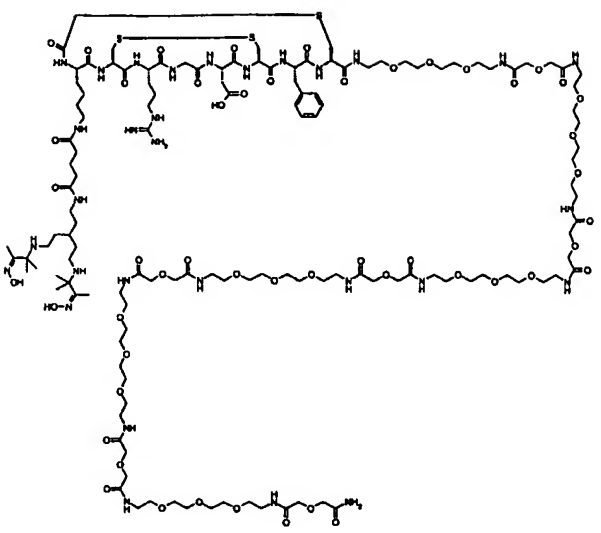
Compound II



Compound III



Compound IV



22. A pharmaceutical composition comprising an effective amount of a compound of general Formula (I) or a salt thereof, together with one or more pharmaceutically acceptable adjuvants, excipients or diluents for use in enhancing image contrast in in vivo imaging or for treatment of a disease.

23. Use of a compound as claimed in any one of claims 1 to 21 in the preparation of a contrast medium for use in a method of diagnosis involving administering said contrast medium to a human or animal body and generating an image of at least part of said body.

24. A method of generating images of a human or animal body involving administering a contrast agent to said body, and generating an image of at least a part of said body to which said contrast agent has distributed, characterised in that said contrast agent comprises a compound as claimed in any one of claims 1 to 21.

25. A method of generating enhanced images of a human or animal body previously administered with a contrast agent composition comprising a compound as claimed in claim 1, which method comprises generating an image of at least part of said body.

26. A method of monitoring the effect of treatment of a human or animal body with a drug to combat a condition associated with cancer, said method involving administering to said body a compound or composition as claimed in any one of claims 1 to 22 and detecting the uptake of said compound or composition by cell receptors, said administration and detection optionally but preferably being effected repeatedly, e.g. before, during and after treatment with said compound or composition.

27. A method of treating cancer or a related disease in a human or animal body which comprises the administration of an effective amount of a compound or composition as claimed in any one of claims 1 to 22.

28. Use of a compound as claimed in any one of claims 1 to 12 for the manufacture of a medicament for the therapeutic or prophylactic treatment of cancer or a related disease in a human or animal.

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(72) Inventors; and

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(57) Abstract: The invention relates to new peptide-based compounds for use as diagnostic imaging agents or as therapeutic agents wherein the agents comprise targeting vectors which bind to integrin receptors.

INTERNATIONAL SEARCH REPORT

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 01 77145 A (INDREVOLL BAARD ; CUTHBERTSON ALAN (NO); NYCOMED IMAGING AS (NO)) 18 October 2001 (2001-10-18) examples claims -/--	1-17, 20-26

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 October 2003	10/11/2003

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax (+31–70) 340–3016	Dullaart, A

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/WO 02/00250

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	RAJOPADHYE M ET AL: "Synthesis, evaluation and Tc-99m complexation of a hydrazinonicotinyl conjugate of a gp IIb/IIIa antagonist cyclic peptide for the detection of deep vein thrombosis" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 7, no. 8, 22 April 1997 (1997-04-22), pages 955-960, XP004136163 ISSN: 0960-894X abstract figures tables 1,2	1-17, 20-26
X	RAJOPADHYE M ET AL: "Synthesis and technetum-99M labeling of cyclic GP IIB/IIIA receptor antagonists conjugated to 4,5-bis(mercaptoacetamido)-pentanoic acid (MAPT)" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 6, no. 15, 6 August 1996 (1996-08-06), pages 1737-1740, XP004135593 ISSN: 0960-894X abstract figures page 1739	1-17, 20-26
X	US 5 888 474 A (LISTER-JAMES JOHN ET AL) 30 March 1999 (1999-03-30) examples claims	1-17, 20-26
X	PEARSON D A ET AL: "THROMBUS IMAGING USING TECHNETIUM-99M-LABELED HIGH-POTENCY GPIIB/IIIA RECEPTOR ANTAGONISTS. CHEMISTRY AND INITIAL BIOLOGICAL STUDIES" JOURNAL OF MEDICINAL CHEMISTRY, AMERICAN CHEMICAL SOCIETY. WASHINGTON, US, vol. 39, no. 7, 1996, pages 1372-1382, XP002061485 ISSN: 0022-2623 schemes 1-5 figure 1 tables 1,2	1-17, 20-26

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INTERNATIONAL SEARCH REPORT

Intern: Application No
PCT/... 02/00250

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HARRIS T D ET AL: "Tc-99m-labeled fibrinogen receptor antagonists: design and synthesis of cyclic RGD peptides for the detection of thrombi" BIOORGANIC & MEDICINAL CHEMISTRY LETTERS, OXFORD, GB, vol. 6, no. 15, 6 August 1996 (1996-08-06), pages 1741-1746, XP004135594 ISSN: 0960-894X abstract figures page 1744, last paragraph - page 1745	1-17, 20-26
X	LIU SHUANG ET AL: "99mTc-labeling of a hydrazinonicotinamide-conjugated vitronectin receptor antagonist useful for imaging tumors" BIOCONJUGATE CHEMISTRY, vol. 12, no. 4, 6 July 2001 (2001-07-06), pages 624-629, XP002259284 & ISSN: 1043-1802 abstract figures tables page 628, left-hand column	1-17, 20-26
X	SIVOLAPENKO G B ET AL: "Imaging of metastatic melanoma utilising a technetium-99m labelled RGD-containing synthetic peptide" EUROPEAN JOURNAL OF NUCLEAR MEDICINE 1998 GERMANY, vol. 25, no. 10, 1998, pages 1383-1389, XP002259285 ISSN: 0340-6997 abstract page 1385, left-hand column, paragraph RESULTS - page 1387, left-hand column	1-17, 20-26
Y	WO 93 12819 A (RHOMED INC) 8 July 1993 (1993-07-08) examples	1-17, 20-26

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INTERNATIONAL SEARCH REPORT

Interna··· l Application No
PCT/NU 02/00250

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	UEHARA T ET AL: "The integrity of the disulfide bond in a cyclic somatostatin analog during <99m>tc complexation reactions - Preparation and preliminary evaluation" NUCLEAR MEDICINE AND BIOLOGY, ELSEVIER SCIENCE PUBLISHERS, NEW YORK, NY, US, vol. 26, no. 8, November 1999 (1999-11), pages 883-890, XP004185286 ISSN: 0969-8051 abstract figures 1,2 page 887, paragraph DISCUSSION - page 889	1-17, 20-26
Y	HALLAHAN D E ET AL: "Targeting drug delivery to radiation-induced neoantigens in tumor microvasculature" JOURNAL OF CONTROLLED RELEASE, ELSEVIER SCIENCE PUBLISHERS B.V. AMSTERDAM, NL, vol. 74, no. 1-3, 6 July 2001 (2001-07-06), pages 183-191, XP004297523 ISSN: 0168-3659 abstract page 188, paragraph 3.3 page 188, paragraph DISCUSSION - page 190	1-28
Y	MERRIFIELD R B: "SOLID PHASE PEPTIDE SYNTHESIS. I. THE SYNTHESIS OF A TETRAPEPTIDE" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, AMERICAN CHEMICAL SOCIETY, WASHINGTON, DC, US, vol. 85, 20 July 1963 (1963-07-20), pages 2149-2154, XP000994867 ISSN: 0002-7863 cited in the application the whole document	1-28
P,X	WO 02 20610 A (SRINIVASAN ANANTHACHARI ; ERION JACK L (US); MALLINCKRODT INC (US); SC) 14 March 2002 (2002-03-14) page 5, line 4 - page 6, line 15 examples claims 4,9,14,22	1-11, 18-20, 22,27,28
Y	WO 99 51638 A (FOK KAM F ; SEARLE & CO (US); TJOENG FOE S (US)) 14 October 1999 (1999-10-14) example 4	1-11, 18-20, 22,27,28

INTERNATIONAL SEARCH REPORT

Int tional application No.
PCT/NO 02/00250

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 24-26 are directed to a diagnostic method practised on the human/animal body, and claim 27 to a method of treatment of the human/animal body, a search has been carried out, based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1-17, 20 and 22-26 in part, 18-19 and 27-28
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [X] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-17, 20 and 22-26 in part, 18-19 and 27-28

Present claims 1-20 and 22-28 relate to an extremely large number of possible compounds, as well as to methods using these compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. With regard to the compounds for which Z1 is an antineoplastic agent, as defined in claims 18-19, no support other than their mere mentioning could be found in the present application. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.
Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds prepared in the examples and to those compounds specifically claimed.
In this respect it is pointed out, that the second subject of the objection for lack of unity of invention has not been searched.

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

nation on patent family members

Internat... Application No
PCT/... 02/00250

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